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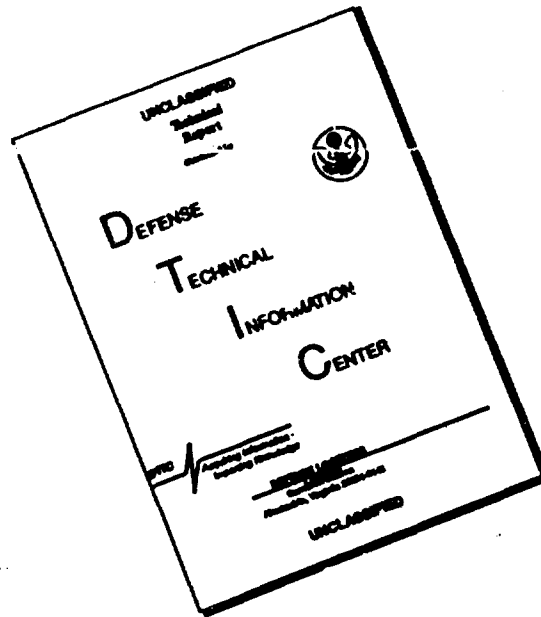
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## RATTUS NORVEGICUS RATS AS POSSIBLE CARRIERS OF ERYSIPELOTOXIN

[Following is the translation of an article by O. V. Ovasapyan, M. M. Yesadzhanyan and V. O. Galoyan, Leninakan Department of the Armenian Antiplague Station, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No 12, 1964, Vol 41, pages 35--38. It was submitted on 15 April 1963. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The first epizootic of erysipeloid in the Leninakan highlands was detected by us in the fall of 1957 among voles and insectivores. Since then this infection has been detected periodically among various species of rodents and ectoparasites.

Somewhat later in Leninakan it was revealed that meat and various equipment at a meat canning plant was contaminated with the erysipeloid causative agent.

According to data from the literature (Vilyavin, 1955, etc.) rodents play a specific role in the spreading of erysipeloid infection, particularly in the meat industry. For the purpose of clearing up the mechanism of transmission and spreading of this infection by rodents we carried out observations on synanthropic rodents, mainly the brown rat.

Over the period of observation (since 1957) we investigated the internal organs of 14,417 grey hamsters and 7,075 house mice. Here it was possible to isolate only individual cultures /5/ of the erysipeloid causative agent. No results at all were obtained from the investigation of the parenchymatous organs of 2,243 brown rats, taken from various places in Leninakan, in spite of the fact that it is primarily the rats which have the greatest contact with meat products and consequently should be infected with the erysipeloid causative agent and be capable of spreading this infection.

The problem arose, do the brown rats fall ill with erysipeloid after the infectious origin enters their body, and does the causative agent penetrate from the digestive tract into the blood and internal organs of the rats, or is it held back in the oral cavity. Since rats are infected most frequently through food it was interesting to clear up how long the erysipeloid causative agent is preserved on the mucous membrane of the pharyngeal ring, and does it multiply on it or does it rapidly disappear.

In a recently published article by Ponomareva et al. we found certain facts which interested us. These concerned the isolation of

erysipelothrix cultures and pasteurilla cultures when investigating washings from the mouths of brown rats. The authors investigated 1,158 brown rats by means of setting up group biological tests. At the same time washings from the mouth and material from the internal organs were used to infect fresh animals (203 biological tests). In this manner erysipelothrix cultures were isolated from 44 rats. From 38 rats they were only from the mouth, from 2 only from the internal organs, and from 4 from both the internal organs and the mouth. The number of cultures isolated is not indicated in the article since each culture was isolated from group biological tests and consequently were related to the several animals included in the group.

One of the missions of our work was to clear up what percentage of rats, inhabiting closed places, are carriers of erysipeloid infection.

From 5 Oct 1962 through 15 Jan 1963 we investigated 134 rats. Of these, 26 were obtained from the Leninakan meat canning plant, 37 from other food objects, 40 in other places in the city (factories, plants, industrial goods warehouses, homes, etc.), and 31 in closed places in the villages of Akhurik and Garibdzhanyan, located 1--2 km from Leninakan.

Each rat was investigated individually by biological and bacteriological methods. The biological test and the bacteriological seeding were carried out simultaneously from the mouth and the internal organs. Using a sterile cotton tampon we took a smear from the depth of the mouth, washed it thoroughly in a small amount (0.7--1 ml) of physiological solution, and inoculated one drop of the washing in a dish of meat-peptone agar (pH 7.3). The remaining suspension was introduced under the skin of a white mouse. Then the rat was dissected and its internal organs inoculated in agar, and a suspension of these organs used to infect white mice.

Thus, for the investigation of 134 brown rats 263 biological probes were set up (parallelly from the mouth and internal organs). As a result 13 cultures of erysipelothrix were isolated, 12 from mouth washings and one from the internal organs (in the last case erysipelothrix was not detected in the mouth washing). All the cultures were isolated only with the help of the biological probe, we were not able to isolate them by a direct seeding from the internal organs (seedings from the mouth usually turned out to be overgrown with various species of microbes).

White mice from which we isolated cultures usually died on the 4--7th day with the typical pathologoanatomical picture of erysipeloid. The remaining test animals (255) were sacrificed on the 15th day and their blood and internal organs were investigated by means of seeding on nutrient media. In spite of a 5-day thorough observation of the dishes the growth of erysipelothrix was not detected.

Of the 26 rats investigated which came from the meat canning plant the erysipeloid causative agent was detected in the mouth of 8 and in the internal organs of one, that is, 9 rats turned out to be infected with erysipeloid. This comprised 34.6% (see the table). At the same

time from the 37 rats which were obtained from other food (non meat) objects 2 carriers of *erysipelo*thrix were detected, and from the 40 obtained from other objects one rat (2.5%) turned out to be infected. Besides those listed, one culture was obtained from the mouth of a rat which came from the village of Garibdzhanyan, where 31 rats were investigated.

Some of the cultures isolated from the mouth of rats (7 strains) were subjected to identification. The cultures did not decompose saccharose and were agglutinated in diagnostic titers (1:800 --1:1600) with the specific serum of *Erysipelothrix rhusiopathiae* var. *suis*. On the basis of this we related the isolated cultures to the *suis* type and identified them with strains of *erysipelo*thrix isolated by us from meat and various equipment in the meat canning plant.

Thus, from an individual investigation of 134 brown rats 12 (around 9%) turned out to be infected with the *erysipeloid* causative agent. This fact served as the basis for proposing the possibility of brown rats as carriers of the *erysipeloid* causative agent.

We conducted special investigations in order to clear up the nature and duration of this carriage. The rats were captured with creels in populated points lying close to Leninakan. At these points the investigation of rodents constantly produced negative results. The animals were kept for 10--15 days under laboratory conditions (each in a separate container). Experimental infection began after a preliminary bacteriological investigation of mouth washings from these rats. Prior to infection not in one case were we able to isolate *erysipelo*thrix, *listeria*, *pasteurella* and other pathogenic microbes.

For the experiment we selected a culture of *Erysipelothrix rhusiopathiae* var. *suis* No 10, isolated from beef, with a minimum lethal dose of 10,000 microbial cells for white mice. All told 9 rats were infected. Three of these received sausage wetted with the culture, the remaining 6 -- half of a corpse of a white mouse which died from *erysipeloid* infection caused by the stated strain. After all the food had been eaten the animals were transferred to clean containers.

We took mouth washings from the rats infected in this manner after 24 hours following infection and then after every 5 days for a period of 25 days. The mouth washings from the experimental rats were investigated by the same method as during the investigation of rats from the separate objects (seedings on agar plates and infection of white mice).

After 24 hours following infection we investigated the mouth washings from 6 rats (infected with the mouse corpses) and isolated a culture of *erysipelo*thrix from 5 of them (the 3 rats which were fed on the sausage were not investigated in the first days). Subsequently, it was possible to isolate *erysipelo*thrix from 3 rats throughout the entire period of investigation, from one a culture of *erysipelo*thrix was isolated up to 10 days, and from the mouths of the other 3 rats the *erysipeloid* causative

agents were isolated only after 24 hours following infection. For the course of the entire experiment diplococci were isolated. The material from the mouths of 2 rats at no time contained erysipelotheix. Pasteurella multocida was isolated from one of these on the 5th day and from the other on the 10th day.

All the enumerated cultures were isolated through white mice, and in single cases we detected a culture of diplococci in direct seedings of mouth washings.

The infected rats were sacrificed after 25 days and investigated by the bacteriological and biological route. Erysipelothrix were not detected either in mouth washings or in the internal organs of these animals.

Both the investigation of rats from various places in Leninakan and the experimental data make it possible to consider that after reaching the mucous membrane of the pharyngeal ring of these animals the erysipeloid causative agent is preserved on it for a certain time and apparently multiplies. In the experimental rats this carriage comprised around 44%. When investigating the mouths of rats captured in the meat canning plant, where there is the greatest possibility of infection from meat products and their wastes, we also detected a significant degree of infection of the mouth with this causative agent (30.7%).

Rats with an oral cavity infected with erysipelotheix may infect healthy meat carcasses. While for the majority of pathogenic microbes multiplication is natural in the tissues and juices of a live organism, the erysipeloid causative agent is capable of multiplying in dead tissues, after an animal has been slaughtered; therefore, they, having entered the meat carcasses, may multiply under the conditions of a meat plant and cause the insemination of these carcasses, which will subsequently further the infection of the remaining meat products and the surrounding furnishings.

On the basis of our previous observations (Ovasapyan, Galoyan) it can be stated that the multiplication of this causative agent is hardly prevented by saprophytic microbes and the low temperature in the refrigerator, and in the presence of an infectious origin its subsequent spreading in meat and other objects is completely possible.

The appearance of Pasteurella multocida and diplococci in the mouths of the experimental rats on the 5-10th day from the onset of the test should be related, as it seems to us, to the fact that individual microbes in doses not sufficient for causing the death of white mice existed on the mucous membrane in the mouths of these rats. Housing of the rats under laboratory conditions favored the multiplication of these microbes. And since pasteurella and diplococci cause the death of mice in earlier periods than erysipelotheix, then this could prevent the isolation of the latter from the mouths of the experimental rats.



### Conclusions

1. At the Leninakan meat canning plant, where it is possible for brown rats to have access to infected meat carcasses, erysipeloid infection of the mucous membrane of the pharyngeal ring in these rodents exceeded 30.7%, while infection of rats taken from other places did not reach 4%.
2. Feeding brown rats material known to be contaminated with a culture of *erysipelothrix* var. *suis* did not cause their death and a clinically expressed disease was not even observed. However, 44% of all the infected animals remained *erysipelothrix* carriers for a certain period of time (10--25 days).
3. Rats, under the conditions present in places dealing with food, may play a specific role in the spreading of erysipeloid infection by the mechanical route and as a result of being carriers. This again emphasizes the problem of the regular and organized eradication of rats.

### Literature

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Results of the laboratory investigation of rats

Places from where the rats were obtained	Number of rats investiga- ted	Number of isolated cultures		Total percentage of infection
		from internal organs	from the mouth	
Meat canning plant	26	1	8	34.6
Other food objects	37	-	2	5.4
Other objects	40	-	1	2.5
Closed places in villages	31	-	1	3.2
Total	134	1	12	9.7